

## EVALUATION OF SOME PLANT ESSENTIAL OILS AS REPELLENT AND TOXICANT AGAINST *TROGODERMA GRANARIUM* (EVERTS) (COLEOPTERA: DERMESTIDAE)

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The essential oils from four medicinal plants viz., *Azadirachta indica*, *Curcuma longa*, *Nigella sativa* and *Piper nigrum* were assessed at a rate of 5, 10, 15 and 20% concentrations against khapra beetle, *Trogoderma granarium* (Everts) for their potential to protect stored wheat grains in term of their repellency and toxicity. Essential oils of *A. indica* gave the highest mean repellency (90.00%) at 20% concentration. The minimum value of repellency (23.56%) was observed in case of *P. nigrum* at 5% concentration. Highest value of mortality (30.68%) was recorded in case of *A. indica*, while minimum value of mortality (14.82%) was observed after 30-days of treatment application at 20% concentration. Similarly, after 5-days of treatment application at 5% concentration, highest mortality was 3.45% in case of *A. indica* and minimum value was 2.24% where application of *P. nigrum* was carried out. Essential oils from these plants caused significant *T. granarium* mortality and deterred the larvae from feeding on wheat grains. Essential oils of these plants could serve as the foundation for development of new botanical insecticides.

**Keywords:** Plant essential oils, *Trogoderma granarium*, Insecticidal and deterrent activity

### INTRODUCTION

The storage of grains and other food products in respect to insect infestation is a serious problem throughout the world. Annual post-harvest losses resulting from insect damage, microbial deterioration and other factors are estimated to be 10-25% of production worldwide (Matthews, 1993). Among various contributing factors to the huge losses, insect pests are a major problem in stored grain throughout the world (Madrid *et al.*, 1990; Sinha and Watters, 1985). In Pakistan, it has been estimated that 5-7% loss of food grain occurs due to the infestation caused by storage insect pests (Jilani and Ahmad, 1982).

Among the insects attacking stored products, *Trogoderma granarium* (Everts) also known as Khapra beetle is one of the most notorious pest of stored grains (Bank, 1977; Hill, 1983) and has attracted the attention of many scientists not only because it can easily be manipulated but also because of the economic importance it has. Losses caused by *T. granarium* have been reported to range from 0.2-2.9% over a period of 1-10.5 months in Pakistan (Irshad *et al.*, 1988). Severe infestation by khapra beetle makes grain unpalatable and unmarketable due to depletion of specific nutrients (Jood and Kapoor, 1994).

In Pakistan, *T. granarium* has been observed to have got resistance against phosphine due to substandard techniques of fumigation (Irshad and Iqbal, 1994). Sharma and Kalra (1998) also reported resistance to phosphine in different stages of *T. granarium*.

Due to the serious problems of genetic resistance by insect species, pest resurgence, residual toxicity, photo toxicity, vertebrate toxicity (Talukder and Howse, 1993; Glenn *et al.*, 1994; Elhang, 2006), and with the increasing cost of synthetic products and the current lack of effective pesticides for stored product protection, evaluation of local plants as sources of protectants is very desirable to help farmers use locally available and environmentally friendly products to limit post-harvest losses of their produce (Bouda *et al.*, 2001). Essential oils are among the best known substances tested for the control of stored product pests. These plant derived compounds may act as repellents (Saim and Meon, 1986; Ndungu *et al.*, 1995; Plarre *et al.*, 1997), contact insecticides (Weaver *et al.*, 1994; Schmidt and Streloke, 1994), antifeedants (Harwood *et al.*, 1990), fumigants (Shaaya *et al.*, 1997) and may affect some biological parameters such as life span, growth rate and reproduction (Stamopoulos, 1991; Pascual-Villalobos, 1996). Plant origin products are receiving greater attention as prophylactic measures against stored product pests, mainly because of their safety to non-target organisms (Jood *et al.*, 1996).

In the light of above said information, present study has been planned to assess the mortality and repellent effects of essential oils of *Azadirachta indica*, *Curcuma longa*, *Nigella sativa* and *Piper nigrum* against *Trogoderma granarium*.

## MATERIALS AND METHODS

**Culture of *Trogoderma granarium*:** Heterogenous population of *T. granarium* (Everts) was collected from various godowns of Punjab Food Department located in Faisalabad, for rearing the test insect population in laboratory. The insects were retained in plastic jars in the laboratory for two months, to get homogenous population of the test insect. The *T. granarium* adults were separated and retained in plastic jars in a medium of wheat grains for breeding purpose, for a period of three days (72 hours) at  $30\pm 2^{\circ}\text{C}$  and  $65\pm 5\%$  R.H. in an incubator. After this insects were taken out from these plastic jars retaining the media (wheat grains) with sufficient number of eggs laid by females. Progeny which was obtained from these rearing plastic jars was of same size and age, which was kept there for another period of one week before testing.

**Preparation of plant essential oils:** Fresh leaves of *Azadirachta indica*, *Piper nigrum* and fruit of *curcuma longa* and *Nigella sativa* were collected from plant growing in the Botanical garden of the University of Agriculture and local market of Faisalabad, respectively. The plant materials were washed in water and then after shade drying these plant materials were ground to bring these in the form of powder. Their extraction was done by using Soxhlet's Extraction Apparatus by dipping 50gm of powder in 250ml acetone; following procedure was described by Valladares *et al.* (1997) and Ahmad *et al.* (2006). Chemical extracts obtained was put in clean and air tight lid bottles. The samples were stored in the refrigerator at  $4^{\circ}\text{C}$  before use.

**Bioassay Studies of Plant Essential Oils against Grubs of *Trogoderma granarium*:** For mortality bioassay 60gm of wheat grains was sprayed with each concentration of test solution uniformly with the help of a pipette. Grains were kept for 60 minutes to allow solvent to evaporate before the start of bioassay. These treated Grains were transferred in plastic jars. One jar was kept as control in each treatment containing untreated grains. Thirty larvae of 3<sup>rd</sup> instar larvae were separately transferred to each plastic jar. After the release of grubs, the mouth of jars were covered with muslin cloth and kept at  $30\pm 2^{\circ}\text{C}$  and  $65\pm 5\%$  R.H. The data regarding %age mortality was recorded after 5, 10, 15 and 30 days of the treatment application. There were 5 treatments with 3 replications in Completely Randomized Design (CRD). The corrected mortality was calculated by Abbott's Formula (1925).

**Repellent effect:** To check the repellent action of these biocides, Jilani and Saxena (1990) experimental method was followed with modification. Whatman No. 1 filter papers (diameter 9cm) were used to check the repellent action. The concentrations of test material were obtained by taking 5, 10, 15 and 20ml of extracts in acetone as solvent to make % age solution. The test solution was applied with micropipette to each half a filter paper disc. The control half was treated with acetone only. In order to evaporate the

solvent from treated and untreated half discs, these filter paper were air dried. Then these halves were attached with each other by using squash tape and kept in Petri plates. Thirty larvae (7-9 days old) of mixed sex were released separately at the center of each filter paper disc. The dishes were then covered and placed in incubator. Three replications were used for each concentration. Observations on the number of insects present on both the treated and untreated halves were recorded after 24 hours.

**Statistical analyses:** Abbott's formula (Abbott, 1925) was used for corrected mortality and data so obtained was analyzed by analysis of variance (ANOVA) using proper statistical package. Tukey's HSD test was also carried out for post hoc testing. A 5% level of significant was used for all statistical tests.

## RESULTS

Repellent action of essential oils of *A. indica*, *C. longa*, *N. sativa* and *P. nigrum* was assessed at four concentrations, viz., 5, 10, 15 and 20% against the grubs of *T. granarium*. The results showed that highest repellency (90.00%) was noted at highest concentration 20% in case of *A. indica*, followed by *C. longa* (81.21%), *N. sativa* (68.57%) and *P. nigrum* (60.00%) respectively (Table 1). The repellency was minimum at lowest concentration (5%) with values 45.37 (*A. indica*), 36.75 (*C. longa*), 31.52 (*N. sativa*) and 23.56% (*P. nigrum*), respectively.

While dealing with respect to individual plant, the repellency value in case of *A. indica* was 90.00% at highest concentration (20%), followed by 15, 10 and 5% with mean values 74.63, 56.42 and 45.37% respectively (Table 1). Similarly, in *C. longa* treatment the repellency was 81.21, 64.82, 48.89 and 36.75% at 20, 15, 10 and 5% concentrations, respectively. In *N. sativa* treatment highest repellency was 68.57% at 20% concentration and minimum (31.52%) at 5% concentration. The repellency in case of *P. nigrum* was 60.00% at 20% concentration, followed by 15, 10 and 5% concentration with values 46.74, 36.67 and 23.56%, respectively. The interaction of plant and concentration gave highest (90.00%) repellency at 20% concentration in case of *A. indica*. It mean that concentration have direct effect on repellency.

The results showed that mortality of *T. granarium* grubs increased with the increasing the concentration and exposure period up to thirty days of treatment application (Table 2). In case of *A. indica* treatment the highest mortality (30.68%) was recorded at 20% concentration and was minimum (13.90%) at 5% concentration after 30<sup>th</sup> day of treatment application. While after 5<sup>th</sup> day of treatment application the mortality was 13.34 and 3.45% at 20 and 5% concentrations, respectively. In *C. longa* treatment the mean mortality was 10.02 and 23.97% after 5<sup>th</sup> and 30<sup>th</sup> day at 20% concentration. While at 5% concentration the mortality was 5.68% after 5<sup>th</sup> day and 11.13% after 30<sup>th</sup> day of treatment application. Similar kind of trend was observed in case of *N. sativa* and *P. nigrum* essential oil treatment with

**Table 1: Repellent effect of different concentrations of plant essential oils of *A. indica*, *C. longa*, *N. sativa* and *P. nigrum* against *T. granarium***

Treatments	Repellency (%) $\pm$ SE			
	<i>A. indica</i>	<i>C. longa</i>	<i>N. sativa</i>	<i>P. nigrum</i>
20	90.00 $\pm$ 1.34 a	81.21 $\pm$ 1.12 a	68.57 $\pm$ 0.78 a	60.00 $\pm$ 1.24 a
15	74.63 $\pm$ 1.57 b	64.82 $\pm$ 0.86 b	52.18 $\pm$ 1.06 b	46.74 $\pm$ 1.08 b
10	56.42 $\pm$ 1.42 c	48.89 $\pm$ 1.64 c	42.84 $\pm$ 1.64 bc	36.67 $\pm$ 1.32 b
5	45.37 $\pm$ 1.20 d	36.75 $\pm$ 1.03 d	31.52 $\pm$ 1.12 c	23.56 $\pm$ 0.86 c

**Table 2: Comparative effect of different concentrations of essential oils of *A. indica*, *C. longa*, *N. sativa* and *P. nigrum* on mortality of *T. granarium* at various exposure periods**

Treatments	Concentrations (%)	Mortality (%) $\pm$ SE			
		5-days	10-days	15-days	30-days
<i>A. indica</i>	20	13.34 $\pm$ 0.73a	17.97 $\pm$ 1.16a	21.84 $\pm$ 1.14a	30.68 $\pm$ 1.52a
	15	8.86 $\pm$ 0.89ab	14.59 $\pm$ 1.38ab	17.09 $\pm$ 1.78a	23.56 $\pm$ 1.65b
	10	5.56 $\pm$ 0.81b	10.07 $\pm$ 1.46bc	15.79 $\pm$ 1.42ab	18.78 $\pm$ 1.38c
	5	3.45 $\pm$ 1.54b	5.03 $\pm$ 1.28c	9.19 $\pm$ 1.34b	13.90 $\pm$ 1.46d
<i>C. longa</i>	20	10.02 $\pm$ 1.42a	14.46 $\pm$ 1.13a	17.78 $\pm$ 1.81a	23.97 $\pm$ 1.52a
	15	7.34 $\pm$ 1.06ab	11.87 $\pm$ 1.32ab	14.02 $\pm$ 1.57ab	19.34 $\pm$ 1.64b
	10	6.36 $\pm$ 1.35bc	8.89 $\pm$ 1.69ab	12.26 $\pm$ 1.69bc	15.62 $\pm$ 1.60c
	5	5.68 $\pm$ 1.20c	5.57 $\pm$ 1.24c	9.23 $\pm$ 1.67c	11.13 $\pm$ 1.86d
<i>N. sativa</i>	20	9.14 $\pm$ 1.08a	12.23 $\pm$ 1.20a	15.68 $\pm$ 1.64a	18.30 $\pm$ 1.58a
	15	6.64 $\pm$ 0.94ab	8.85 $\pm$ 1.43b	11.24 $\pm$ 1.65b	15.97 $\pm$ 1.64a
	10	4.47 $\pm$ 1.24bc	6.72 $\pm$ 1.68bc	8.61 $\pm$ 1.06bc	11.86 $\pm$ 1.65b
	5	1.83 $\pm$ 1.34c	4.62 $\pm$ 1.42c	5.67 $\pm$ 1.43c	8.89 $\pm$ 1.76c
<i>P. nigrum</i>	20	5.56 $\pm$ 1.45a	8.89 $\pm$ 1.57a	11.78 $\pm$ 1.65a	14.82 $\pm$ 1.46a
	15	4.54 $\pm$ 1.02a	7.78 $\pm$ 1.45a	8.85 $\pm$ 1.38b	10.87 $\pm$ 1.58b
	10	2.73 $\pm$ 1.24a	4.45 $\pm$ 1.34ab	4.46 $\pm$ 1.56c	8.27 $\pm$ 1.45c
	5	2.24 $\pm$ 1.56a	2.68 $\pm$ 1.54b	3.79 $\pm$ 1.43d	5.39 $\pm$ 1.48d

respect to concentration and exposure period. The mortality value was 18.30 and 8.89% at 20 and 5% concentrations, respectively after 30<sup>th</sup> day of treatment application of *N. sativa*. After 5<sup>th</sup> day the mortality was 9.14 and 1.83% at 20 and 5% concentrations. In *P. nigrum* treatment the mortality value with respect to exposure period at 20% concentration was 5.56% after 5<sup>th</sup> day and 14.82% after 30<sup>th</sup> day of application. Similarly at 5% concentration mortality was 2.24 and 5.39% after 5<sup>th</sup> and 30<sup>th</sup> day of treatment application, respectively Table 2).

Overall results revealed that *A. indica* was the most effectively plant among all the test plants under study, both in term of repellency and mortality. It was also observed that both repellency and mortality was increased with the increase in concentration, so there is direct dose response relationship.

## DISCUSSION

Similar findings of repellent effect were described by the other scientists with *Tribolium castaneum* and *Sitophilus zeamais* treated with Pepper and Garlic extracts (Chew and Ho, 2000). Essential oils from *Piper guineense* against weevils gave the similar results (Keita *et al.*, 2000). Our results were also coincide with those of Jilani *et al.* (2003), Dwivedi and Shekhawat (2004), Rehman *et al.* (2007), Khanam *et al.* (2008). Our findings regarding repellency was also in agreement with those of Sagheer *et al.* (2013), who study the repellent effect of four medicinal plants

against *T. granarium*. They also observed that a direct dose response relationship between concentration and repellency. The results of the present study regarding mortality was in agreement with those of Chew and Ho (2000), Sighamony *et al.* (2007), Mahdi and Rehman (2008) who reported the effectiveness of Pepper extracts for the control of storage pests. Paul *et al.* (2009) reported the toxic effect of oil and extracts against different stored product pests, which were matched to our findings. Kumar (2007) reported similar findings by using *Nigella sativa* against *Tribolium castaneum*. Our results were also in pipeline with Hasan *et al.* (2013) who tested different plant powders, *Nigella sativa* was one of them for the management of *Callosobruchus maculatus*. They observed that the *N. sativa* showed highest mortality effect against *C. maculatus* than all the other test plant powders.

According to the above said authors, plant essential oils have repellent and toxic effects against the number of stored grain insect pests.

## CONCLUSION

Keeping in view the above results, it is suggested that we should give more attention to develop commercial botanical insecticides which are safe for health as well as environment, insecticides which will not only control the insect pests of stored products but also safe for health and environment because storage is our main objective. The information about plant extracts will serve as guideline for planning cost and adopting effective measure against *T. granarium*.

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